VERIFICATION

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hereby declare as follows:

1. I am familiar with both the English and Japanese languages.

2. I certify that the following is a true translation of Japanese Patent Application No. 6412/1998 filed on January 16, 1998.

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Signature of translator: 

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[Name of Document] Specification
[Title of the Invention]SUSTAINED-RELEASE COMPOSITION, METHOD OF ITS PRODUCTION AND USE THEREOF
[Claims]
[Claim 1] A sustained-release composition containing a hydroxynaphthoic acid salt of a biologically active substance.
[Claim 2] The sustained-release composition according to claim 1 wherein a biodegradable polymer is further contained.
[Claim 3] The sustained-release composition according to claim 1 or 2 wherein said biologically active substance is a biologically active peptide.
[Claim 4] The sustained-release composition according to claim 3 wherein said biologically active peptide is an LH-RH derivative.
[Claim 5] The sustained-release composition according to claim 1 or 2 wherein said hydroxynaphthoic acid is 3-hydroxy-2-naphthoic acid.
[Claim 6] The sustained-release composition according to claim 2 wherein said biodegradable polymer is an α-hydroxycarboxylic acid polymer.
[Claim 7] The sustained-release composition according to claim 6 wherein said α-hydroxycarboxylic acid polymer is a lactic acid-glycolic acid polymer.
[Claim 8] The sustained-release composition according to claim 7 wherein the content ratio of lactic acid and glycolic acid is 100/0 to 40/60 mol%.
[Claim 9] The sustained-release composition according to claim 8 wherein the weight-average molecular weight of the polymer is about 3,000 to about 100,000.
[Claim 10] The sustained-release composition according to claim 1 which is slightly soluble in water.
[Claim 11] The sustained-release composition according to claim 1 or 2 intended for injection use.

[Claim 12] A production method for the sustained-release composition according to claim 1 comprising removing the solvent from the mixture containing the hydroxynaphthoic acid salt of the biologically active substance.
[Claim 13] A production method for the sustained-release composition according to claim 2 comprising removing the solvent from the mixture of the biologically active substance, biodegradable polymer and hydroxynaphthoic acid or salt thereof.
[Claim 14] The production method according to claim 12 or 13 wherein said biologically active peptide is a salt of a free base or acid.
[Claim 15] A pharmaceutical containing the sustained-release composition according to claim 1 or 2.
[Claim 16] A prophylactic/therapeutic agent for prostatic cancer, prostatic hypertrophy, endometriosis, hysteromyoma, metrofibroma, precocious puberty or breast cancer, or contraceptive, containing the sustained-release composition according to claim 4.
[Claim 17] A sustained-release composition containing a biologically active substance, a hydroxynaphthoic acid or salt thereof, and a biodegradable polymer.

Detailed Description of the Invention

[Field of industrial application]

The present invention relates to a sustained-release composition of a biologically active substance, a production method therefor, and its use as a pharmaceutical etc.

[Prior art]

Japanese Patent Unexamined Publication No. 97334/1995 discloses a sustained-release preparation comprising a biologically active peptide or salt thereof and a biodegradable polymer having a free carboxyl group at one end, and

a production method therefor.

The patent publications for GB2209937, GB2234169, GB2234896, GB2257909 and EP626170A2 disclose compositions based on a biodegradable polymer containing a separately prepared water-insoluble salt, such as a pamoate of a peptide or protein, or production methods therefor.

The patent publication for WO95/15767 discloses the embonate (pamoate) of cetrorelix (LH-RH antagonist) and a production method therefor, and describes that the peptide-releasing profile of this pamoate remains the same as in its use alone, even when included in a biodegradable polymer.

[Problems to be solved by the invention]

To provide a novel composition that contains a biologically active substance at high contents, and that is capable of controlling the rate of its release.

[Means of solving the problems]

After extensive investigation aiming at resolving the above problem, the present inventors found that when the biologically active substance is incorporated at high contents in the composition by allowing the biologically active substance and the hydroxynaphthoic acid to be co-present during formation of the composition, and when both are included in the biodegradable polymer, the biologically active substance is released at rates differing from those of the biologically active substance from the counterpart composition of the biologically active substance and hydroxynaphthoic acid prepared in the absence of the biodegradable polymer, which rate of release being controllable by choosing the appropriate kind of biodegradable polymer. The inventors conducted further investigation based on this finding, and developed the present invention.

Accordingly, the present invention provides:

(1) a sustained-release composition containing a hydroxynaphthoic acid salt of a biologically active substance,

(2) the sustained-release composition according to term (1) above wherein a biodegradable polymer is further contained,

(3) the sustained-release composition according to term (1) or (2) above wherein said biologically active substance is a biologically active peptide,

(4) the sustained-release composition according to term (3) above wherein said biologically active peptide is an LH-RH derivative,

(5) the sustained-release composition according to term (1) or (2) above wherein said hydroxynaphthoic acid is 3-hydroxy-2-naphthoic acid,

(6) the sustained-release composition according to term (2) above wherein said biodegradable polymer is an α-hydroxycarboxylic acid polymer,

(7) the sustained-release composition according to term (6) above wherein said α-hydroxycarboxylic acid polymer is a lactic acid-glycolic acid polymer,

(8) the sustained-release composition according to term (7) above wherein the content ratio of lactic acid and glycolic acid is 100/0 to 40/60 mol%,

(9) the sustained-release composition according to term (8) above wherein the weight-average molecular weight of the polymer is about 3,000 to about 100,000,

(10) the sustained-release composition according to term (1) above which is slightly soluble in water,

(11) the sustained-release composition according to term (1) or (2) above which is intended for injection use,

(12) a production method for the sustained-release composition according to term (1) above comprising removing the solvent from the mixture containing the hydroxynaphthoic acid salt of the biologically active substance,

(13) a production method for the sustained-release composition according to term (2) above comprising removing the solvent from the mixture of the biologically active substance, biodegradable polymer and hydroxynaphthoic acid or salt thereof,
(14) the production method according to term (12) or (13) above wherein said biologically active peptide is a salt of a free base or acid,
(15) a pharmaceutical containing the sustained-release composition according to term (1) or (2) above,
(16) a prophylactic/therapeutic agent for prostatic cancer, prostatic hypertrophy, endometriosis, hysteromyoma, metrofibroma, precocious puberty or breast cancer, or contraceptive, containing the sustained-release composition according to term (4) above, and
(17) a sustained-release composition containing a biologically active substance, a hydroxynaphthoic acid or salt thereof, and a biodegradable polymer.

The present invention further provides:
(18) the sustained-release composition according to term (1) above wherein the hydroxynaphthoic acid content is about 1 to about 7 mol, preferably about 1 to about 2 mol, per mol of biologically active substance,
(19) the sustained-release composition production method according to term (13) above comprising mixing and dissolving a biologically active substance or salt thereof and a hydroxynaphthoic acid or salt thereof, then removing the solvent,
(20) the sustained-release composition production method according to term (14) above comprising dissolving a composition of a biologically active substance or salt thereof and a hydroxynaphthoic acid or salt thereof in a solution of a biodegradable polymer in an organic solvent, and removing the solvent,
(21) the sustained-release composition production method

according to term (14) above comprising mixing and dissolving a biologically active substance or salt thereof, a biodegradable polymer, and a hydroxynaphthoic acid or salt thereof, then removing the solvent,
(22) the sustained-release composition production method according to term (14) above comprising producing a W/O emulsion with a solution containing a biologically active substance or salt thereof as an internal water phase and a solution containing a biodegradable polymer and a hydroxynaphthoic acid or salt thereof as an oil phase, then removing the solvent,
(23) the sustained-release composition production method according to term (14) above comprising producing a W/O emulsion with a solution containing a hydroxynaphthoic acid or salt thereof as an internal water phase and a solution containing a biologically active substance or salt thereof and a biodegradable polymer as an oil phase, then removing the solvent, and
(24) the sustained-release composition production method according to any one of terms (20) through (23) above wherein the method of solvent removal is the aqueous drying method.

Although the biologically active substance used in the present invention is not subject to limitation, as long as it is pharmacologically useful, biologically active peptides, for example, are preferred, particularly those having molecular weights of about 300 to about 40,000, preferably about 400 to about 30,000, and more preferably about 500 to about 20,000.

Such biologically active peptides include, for example, luteinizing hormone-releasing hormone (LH-RH), insulin, somatostatin, growth hormones, growth hormone-releasing hormone (GH-RH), prolactin, erythropoietin, adrenocorticotropic hormone, melanocyte-stimulating hormone, thyroid hormone-releasing hormone, thyroid-

stimulating hormone, luteinizing hormone, follicle-stimulating hormone, vasopressin, oxytocin, calcitonin, gastrin, secretin, pancreozymin, cholecystokinin, angiotensin, human placental lactogen, human chorionic gonadotropin, enkephalin, endorphin, kyotorphin, tuftsin, thymopoietin, thymosin, thymostimulin, thymic humoral factor, blood thymic factor, tumor necrosis factor, colony-stimulating factor, motilin, daynorphin, bombesin, neurotensin, caerulein, bradykinin, atrial natriuresis-increasing factor, nerve growth factor, cell growth factor, neurotrophic factor, endothelin-antagonistic peptides, derivatives thereof, fragments of these peptides, and derivatives of such fragments.

The biologically active peptide used in the present invention may be as is, or may be a pharmacologically acceptable salt. Such salts include salts with inorganic acids (e.g., hydrochloric acid, sulfuric acid, nitric acid, boric acid), organic acids (e.g., carbonic acid, bicarbonic acid, succinic acid, acetic acid, propionic acid, trifluoroacetic acid) etc., when said biologically active peptide has a basic group such as an amino group.

When said biologically active peptide has an acidic group such as a carboxyl group, such salts include salts with inorganic bases (e.g., alkali metals such as sodium and potassium, alkaline earth metals such as calcium and magnesium), organic bases (e.g., organic amines such as triethylamine, basic amino acids such as arginine) etc. The biologically active peptide may form a metal complex compound (e.g., copper complex, zinc complex).

Preferred examples of the above-described biologically active peptide are LH-RH derivatives or salts thereof that are effective against sex hormone-dependent diseases such as prostatic cancer, prostatic hypertrophy, endometriosis, hysteromyoma, precocious puberty and breast cancer, and effective for contraception.

Examples of LH-RH derivatives or salts thereof include, for example, the peptides described in "Treatment with GnRH Analogs: Controversies and Perspectives" (The Parthenon Publishing Group Ltd., published 1996), Japanese Patent Examined Publication No. 503165/1991, Japanese Patent Unexamined Publication Nos. 101695/1991, 97334/1995 and 259460/1996, and elsewhere.

LH-RH derivatives may be LH-RH agonists or LH-RH antagonists; useful LH-RH antagonists include, for example, biologically active peptides represented by general formula [I]:

X-D2Nal-D4ClPhe-D3Pal-Ser-A-B-Leu-C-Pro-DAlaNH2

[X represents N(4H2-furoyl)Gly or NAc; A represents a residue selected from NMeTyr, Tyr, Aph(Atz) and NMeAph(Atz); B represents a residue selected from DLys(Nic), DCit, DLys(AzaglyNic), DLys(AzaglyFur), DhArg(Et_2), DAph(Atz) AND DhCi; C represents Lys(Nisp), Arg or hArg(Et_2)] or salts thereof.

Useful LH-RH agonists include, for example, biologically active peptides represented by general formula [II]:

5-oxo-Pro-His-Trp-Ser-Tyr-Y-Leu-Arg-Pro-Z

[Y represents a residue selected from DLeu, DAla, DTrp, DSer(tBu), D2Nal and DHis(1mBzl); Z represents Gly-NH_2] or salts thereof. Peptides wherein Y is DLeu and Z is NH-C_2H_5, in particular, are preferred.

These peptides can be produced by the methods described in the above-mentioned references or patent publications, or methods based thereon.

The abbreviations used herein are defined as follows:

Abbreviation	Name
N(4H2-furoyl)Gly	: N-tetrahydrofuroylglycine residue
NAc	: N-acetyl group
D2Nal	: D-3-(2-naphthyl)alanine residue
D4ClPhe	: D-3-(4-chloro)phenylalanine resi-

		due
D3Pal	:	D-3-(3-pyridyl)alanine residue
NMeTyr	:	N-methyltyrosine residue
Aph(Atz)	:	N-[5'-(3'-amino-1'H-1',2',4'-triazolyl)]phenylalanine residue
NMeAph(Atz)	:	N-methyl-[5'-(3'-amino-1'H-1',2',4'-triazolyl)]phenylalanine residue
DLys(Nic)	:	D-(e-N-nicotinoyl)lysine residue
Dcit	:	D-citrulline residue
DLys(AzaglyNic)	:	D-(azaglycylnicotinoyl)lysine residue
DLys(AzaglyFur)	:	D-(azaglycylfuranyl)lysine residue
DhArg(Et2)	:	D-(N,N'-diethyl)homoarginine residue
DAph(Atz)	:	D-N-[5'-(3'-amino-1'H-1',2',4'-triazolyl)]phenylalanine residue
DhCi	:	D-homocitrulline residue
Lys(Nisp)	:	(e-N-isopropyl)lysine residue
hArg(Et2)	:	(N,N'-diethyl)homoarginine residue

The abbreviations for amino acids are based on abbreviations specified by the IUPAC-IUB Commission on Biochemical Nomenclature [European Journal of Biochemistry, Vol. 138, pp. 9-37 (1984)] or abbreviations in common use in relevant fields. When an optical isomer may be present in amino acid, it is of the L-configuration, unless otherwise stated.

The hydroxynaphthoic acid used in the present invention is a compound wherein two different carbons in naphthalene are bound with one hydroxyl group and one carboxyl group. Useful hydroxynaphthoic acids include, for example, 3-hydroxy-2-naphthoic acid, 1-hydroxy-2-naphthoic acid, 2-hydroxy-1-naphthoic acid, and 6-hydroxy-2-naphthoic acid, with preference given to 3-hydroxy-2-naphthoic acid,

wherein the 3-position carbon in naphthalene is bound with a hydroxyl group, and the 2-position carbon with a carboxyl group.

The hydroxynaphthoic acid may be a salt. Salts include, for example, salts with inorganic bases such as alkali metals (e.g., sodium, potassium) and alkaline earth metal salts (e.g., calcium salts, magnesium salts), and salts with transition metals (e.g., zinc, iron, copper).

Biodegradable polymers used in the present invention include, for example, polymers and copolymers that have been synthesized from one or more kinds selected from α-hydroxy acids (e.g., glycolic acid, lactic acid), hydroxydicarboxylic acids (e.g., malic acid), hydroxytricarboxylic acids (e.g., citric acid) etc., and that have a free carboxyl group, or mixtures thereof; poly-α-cyanoacrylic acid esters; polyamino acids (e.g., poly-g-benzyl-L-glutamic acid); and maleic anhydride copolymers (e.g., styrene-maleic acid copolymers).

Polymerization may be of the random, block or graft type. When the above-mentioned α-hydroxy acids, hydroxydicarboxylic acids and hydroxytricarboxylic acids have an optical active center in their molecular structures, they may be of the D-, L- or DL-configuration. Of these, lactic acid-glycolic acid polymers, poly-α-cyanoacrylic acid esters etc. are preferred. Greater preference is given to lactic acid-glycolic acid polymers.

When the biodegradable polymer used is a lactic acid-glycolic acid polymer, the content ratio (mol%) is preferably 100/0 to 30/70, more preferably 100/0 to 40/60.

The weight-average molecular weight of the lactic acid-glycolic acid polymer is normally about 3,000 to about 500,000, preferably about 3,000 to about 200,000, and more preferably about 3,000 to about 100,000. The degree of dispersion (weight-average molecular

weight/number-average molecular weight) is normally about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

The decomposition/elimination rate of a lactic acid-glycolic acid polymer varies widely, depending on composition or molecular weight. However, drug release duration can be extended by lowering the glycolic acid ratio or increasing the molecular weight, because decomposition/elimination is usually delayed as the glycolic acid ratio decreases. Conversely, drug release duration can be shortened by increasing the glycolic acid ratio or decreasing the molecular weight. To obtain a sustained-release preparation of the long acting type (e.g., 1-12 months, preferably 1-6 months), it is preferable to use a lactic acid-glycolic acid polymer whose content ratio and weight-average molecular weight fall in the above ranges. If choosing a lactic acid-glycolic acid polymer that decomposes more rapidly than that whose content ratio and weight-average molecular weight fall in the above ranges, initial burst is difficult to suppress; if choosing a lactic acid-glycolic acid polymer that decomposes more slowly than that whose content ratio and weight-average molecular weight fall in the above ranges, it is likely that no effective amount of drug is released during some period.

Weight-average molecular weight, number-average molecular weight and degree of dispersion, as defined herein, are polystyrene-based molecular weights and degree of dispersion determined by gel permeation chromatography (GPC) with 14 polystyrenes as reference substances with weight-average molecular weights of 1,110,000, 707,000, 354,000, 189,000, 156,055, 98,900, 66,437, 37,200, 17,100, 9,830, 5,870, 2,500, 1,303, and 500, respectively. Measurements were taken using a GPC column KF804Lx2 (produced by Showa Denko) and an RI monitor L-3300 (produced by Hitachi, Ltd.), with chloroform as a mobile phase. Also, number-average molecular weight was calculated by dissolving the

biodegradable polymer in an acetone-methanol mixed solvent, and titrating this solution with an alcoholic solution of potassium hydroxide with phenolphthalein as an indicator, to determine the terminal carboxyl group content. This molecular weight is hereinafter referred to as number-average molecular weight based on terminal group quantitation. While the number-average molecular weight based on terminal group quantitation is an absolute value, that based on GPC measurement is a relative value that varies depending on various analytical conditions (e.g., kind of mobile phase, kind of column, reference substance, slice width, baseline); it is therefore difficult to have an absolute numerical representation of both values. In the case of polymers having a free carboxyl group at one end, that have been synthesized from lactic acid and glycolic acid by the catalyst-free dehydration polymerization condensation method, however, the number-average molecular weights based on GPC measurement and terminal group quantitation almost agree with each other. This fact means that the number-average molecular weight based on terminal group quantitation falls within the range from about 0.2 to about 1.5 times, preferably from about 0.3 to about 1.2 times, that based on GPC measurement.

A lactic acid-glycolic acid polymer can be produced by, for example catalyst-free dehydration polymerization condensation from a lactic acid and a glycolic acid (Japanese Patent Unexamined Publication No. 28521/1986) or ring-opening polymerization from a lactide and a cyclic compound such as glycolide by means of a catalyst (Encyclopedic Handbook of Biomaterials and Bioengineering Part A: Materials, Volume 2, Marcel Dekker, Inc., 1995).

Although the polymer synthesized by ring-opening polymerization is a polymer not having a carboxyl group, it is also possible to use a polymer prepared by chemically treating said polymer to render its terminal a free

carboxyl group [Journal of Controlled Release, Vol. 41, pp. 249-257 (1996)].

The above-described lactic acid-glycolic acid polymer having a free carboxyl group at one end can be produced without any problems by known production methods (e.g., catalyst-free dehydration polymerization condensation method, see Japanese Patent Unexamined Publication No. 28521/1986); a polymer having a free carboxyl group elsewhere (not limited to terminals) can be produced by known production methods (e.g., see Patent Publication for WO94/15587).

Also, the lactic acid-glycolic acid polymer prepared by chemical treatment after ring-opening polymerization to render its terminal a free carboxyl group may be a commercial product of Boehringer Ingelheim KG, for example.

The sustained-release composition of the present invention, which contains a hydroxynaphthoic acid salt of a biologically active substance, may have said biologically active substance and hydroxynaphthoic acid contained in a non-salt form. That is, said sustained-release composition may contain not only the hydroxynaphthoic acid salt of the biologically active substance but also another biologically active substance or salt thereof (hydroxynaphthoic acid salt excluded) and hydroxynaphthoic acid or salt thereof (salt with biologically active substance excluded).

The sustained-release composition of the present invention, which contains a biologically active substance, hydroxynaphthoic acid or salt thereof and biodegradable polymer, may permit the three components to form a salt. For example, the sustained-release composition of the present invention may contain (1) a hydroxynaphthoic acid salt of a biologically active substance and a biodegradable polymer, or (2) a hydroxynaphthoic acid salt of a biologically active substance and a salt formed between a biologically active substance and a biodegradable polymer.

In the sustained-release composition of the present invention, a hydroxynaphthoic acid or salt thereof can serve as a base for the sustained-release composition.

Although it varies depending on kind of biologically active substance, desired pharmacological effect, duration of effect and other factors, the biologically active substance content in the sustained-release composition of the present invention is normally about 5 to about 90% by weight, preferably about 10 to about 85% by weight, more preferably about 20 to about 80% by weight, and still more preferably about 30 to about 80% by weight, relative to the sum of biologically active substance and hydroxynaphthoic acid, when the sustained-release composition of the present invention consists of the two components. The hydroxynaphthoic acid content is normally about 10 to about 95% by weight, preferably about 15 to about 90% by weight, more preferably about 20 to about 80% by weight, and still more preferably about 20 to about 70% by weight, relative to the sum of biologically active substance and hydroxynaphthoic acid.

When the sustained-release composition of the present invention consists of three components, i.e., biologically active substance, hydroxynaphthoic acid and biodegradable polymer, the hydroxynaphthoic acid content in the sustained-release composition of the present invention is normally about 1 to about 60% by weight, preferably about 2 to about 50% by weight, more preferably about 5 to about 45% by weight, and still more preferably about 10 to about 40% by weight, relative to the sum of the three.

The hydroxynaphthoic acid content is normally about 0.1 to about 30% by weight, preferably about 0.2 to about 25% by weight, and more preferably about 0.5 to about 20% by weight, relative to the sum of the three. The biodegradable polymer content is normally about 20 to about 99% by weight, preferably about 30 to about 95% by weight, and

more preferably about 40 to about 90% by weight, relative to the sum of the three.

The content ratio of biologically active substance and hydroxynaphthoic acid or salt thereof in the sustained-release composition of the present invention is normally about 0.1 to about 8 mol of hydroxynaphthoic acid or salt thereof, per mol of biologically active substance. In the case of a sustained-release composition containing two components, i.e., biologically active substance and hydroxynaphthoic acid, in particular, the content ratio is preferably about 1 to about 7 mol, more preferably about 1 to about 2 mol, of hydroxynaphthoic acid or salt thereof, per mol of biologically active substance. In the case of a sustained-release composition containing three components, i.e., biologically active substance, hydroxynaphthoic acid or salt thereof and biodegradable polymer, the content ratio is preferably about 0.1 to about 3 mol, more preferably about 0.2 to about 2 mol, of hydroxynaphthoic acid or salt thereof, per mol of biologically active substance.

Although the sustained-release composition of the present invention is not subject to limitation regarding morphology, microparticle forms are preferred, with greater preference given to microspheres. The term microsphere, as used herein, refers to an injectable spherical microparticle dispersible in solution. Its shape can be confirmed by, for example, scanning electron microscopy.

[Modes of embodiment of the invention]

Production methods for sustained-release compositions of the present invention, which contain a hydroxynaphthoic acid salt of a biologically active substance, e.g., microspheres, are exemplified below.

(I) Two-step method

A biologically active substance or salt thereof is added to a solution of a hydroxynaphthoic acid or salt

thereof in an organic solvent to a weight ratio falling within the above-described content range for biologically active substances, to yield an organic solvent solution of the biologically active substance and the hydroxynaphthoic acid.

Said organic solvent is exemplified by alcohols (e.g., ethanol, methanol), acetonitrile, halogenated hydrocarbons (e.g., dichloromethane, chloroform, dichloroethane, trichloroethane, carbon tetrachloride), ethers (e.g., ethyl ether, isopropyl ether), fatty acid esters (e.g., ethyl acetate, butyl acetate), and aromatic hydrocarbons (e.g., benzene, toluene, xylene). These solvents may be used in mixtures at appropriate ratios. Of these solvents, alcohols are preferred, with greater preference given to ethanol.

Organic solvent removal for precipitation of a composition of a biologically active substance and a hydroxynaphthoic acid can be achieved by commonly known methods or methods based thereon. Such methods include, for example, the method in which the organic solvent is evaporated while the degree of vacuum is adjusted using a rotary evaporator or the like.

The thus-obtained composition of a biologically active substance and a hydroxynaphthoic acid can be again dissolved in an organic solvent to yield a sustained-release composition (microspheres or microparticles).

Said organic solvent is exemplified by halogenated hydrocarbons (e.g., dichloromethane, chloroform, dichloroethane, trichloroethane, carbon tetrachloride), ethers (e.g., ethyl ether, isopropyl ether), fatty acid esters (e.g., ethyl acetate, butyl acetate), and aromatic hydrocarbons (e.g., benzene, toluene, xylene). These solvents may be used in mixtures at appropriate ratios. Of these solvents, halogenated hydrocarbons are preferred, with greater preference given to dichloromethane.

The organic solvent solution containing the biologically active substance and hydroxynaphthoic acid is then added to a water phase to form an O (oil phase)/W (water phase) emulsion, after which the solvent is evaporated from the oil phase to yield microspheres. For this operation, the water phase volume is normally chosen over the range from about 1 time to about 10,000 times, preferably from about 5 times to about 5,000 times, and more preferably from about 10 times to about 2,000 times, the oil phase volume.

An emulsifier may be added to the above-described external water phase. Said emulsifier may be any one, as long as it is capable of forming a stable O/W emulsion. Such emulsifiers include, for example, anionic surfactants (e.g., sodium oleate, sodium stearate, sodium lauryl sulfate), nonionic surfactants [e.g., polyoxyethylene sorbitan fatty acid esters (Tween 80, Tween 60, Atlas Powder Company), polyoxyethylene castor oil derivatives (e.g., HCO-60, HCO-50, Nikko Chemicals)], polyvinylpyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin and hyaluronic acid. These emulsifiers may be used singly or in combination. Regarding the concentration, it is preferable that they be used over the range from about 0.01 to 10% by weight, preferably from about 0.05 to about 5% by weight.

An osmotic pressure regulator may be added to the above-described external water phase. The osmotic pressure regulator used in the present invention may be any one, as long as it shows an osmotic pressure when prepared as an aqueous solution. Said osmotic pressure regulator is exemplified by polyhydric alcohols, monohydric alcohols, monosaccharides, disaccharides, oligosaccharides, and derivatives thereof.

Useful polyhydric alcohols include, for example, dihydric alcohols such as glycerol, pentahydric alcohols

such as arabitol, xylitol and adonitol, and hexahydric alcohols such as mannitol, sorbitol and dulcitol. Of these alcohols, hexavalent alcohols etc. are preferred, with greater preference given to mannitol.

Useful monohydric alcohols include, for example, methanol, ethanol and isopropyl alcohol, with preference given to ethanol.

Useful monosaccharides include, for example, pentoses such as arabinose, xylose, ribose and 2-deoxyribose, and hexoses such as glucose, fructose, galactose, mannose, sorbose, rhamnose and fucose, with preference given to pentoses etc.

Useful oligosaccharides include, for example, trisaccharides such as maltotriose and raffinose, and tetrasaccharides such as stachyose, with preference given to trisaccharides.

Useful derivatives of monosaccharides, disaccharides and oligosaccharides include, for example, glucosamine, galactosamine, glucuronic acid and galacturonic acid.

These osmotic pressure regulators are normally used at such concentrations that the external water phase osmotic pressure is about 1/50 to about 5 times, preferably about 1/25 to about 3 times, the physiological saline osmotic pressure.

Organic solvent removal can be achieved by commonly known methods or methods based thereon. Such methods include, for example, the method in which the organic solvent is evaporated under normal or gradually reduced pressure during stirring using a propeller stirrer, magnetic stirrer or the like, and the method in which the organic solvent is evaporated while the degree of vacuum is adjusted using a rotary evaporator or the like.

The thus-obtained microspheres are centrifuged or filtered to separate them, after which they are washed with distilled water several times to remove the free bi-

ologically active substance, hydroxynaphthoic acid, emulsifier etc. adhering to the microsphere surface, then again dispersed in distilled water etc. and freeze-dried.

To prevent mutual aggregation of particles during the production process, an anticoagulant may be added. Said anticoagulant is exemplified by water-soluble polysaccharides such as mannitol, lactose, glucose and starches (e.g., corn starch), amino acids such as glycine, and proteins such as fibrin and collagen. Of these substances, mannitol is preferred.

Where necessary, freeze-drying may be followed by heating under reduced pressure without causing mutual adhesion of microspheres, to further remove the water and organic solvent from the microspheres.

Although it varies depending on the amount of microspheres and other factors, heating time is normally about 12 hours to about 168 hours, preferably about 24 hours to about 120 hours, and more preferably about 48 hours to about 96 hours, after the microspheres reach a given temperature.

Any heating method can be used, as long as microsphere aggregates are uniformly heated.

Useful thermal drying methods include, for example, the method in which thermal drying is conducted in a constant-temperature chamber, fluidized bed chamber, mobile chamber or kiln, and the method using microwaves for thermal drying. Of these methods, the method in which thermal drying is conducted in a constant-temperature chamber is preferred. The microspheres obtained are relatively uniformly spherical and undergo little resistance during administration by injection so that needle clogging is unlikely. Also, possible use of thin injection needles mitigates patient pain at injection.

(II) One-step method

A biologically active substance or salt thereof is

added to a solution of a hydroxynaphthoic acid or salt thereof in an organic solvent to a weight ratio falling within the above-described content range for biologically active substances, to yield an organic solvent solution of the biologically active substance and the hydroxynaphthoic acid, after which a sustained-release preparation (microspheres or microparticles) is prepared.

Said organic solvent is exemplified by alcohols (e.g., ethanol, methanol), acetonitrile, halogenated hydrocarbons (e.g., dichloromethane, chloroform, dichloroethane, trichloroethane, carbon tetrachloride), ethers (e.g., ethyl ether, isopropyl ether), fatty acid esters (e.g., ethyl acetate, butyl acetate), and aromatic hydrocarbons (e.g., benzene, toluene, xylene). These solvents may be used in mixtures at appropriate ratios. Of these solvents, mixtures of halogenated hydrocarbons and alcohols are preferred, with greater preference given to mixtures of dichloromethane and ethanol.

The organic solvent solution containing the biologically active substance and hydroxynaphthoic acid is then added to a water phase to form an O (oil phase)/W (water phase) emulsion, after which the solvent is evaporated from the oil phase to yield microspheres. For this operation, the water phase volume is normally chosen over the range from about 1 time to about 10,000 times, preferably from about 5 times to about 5,000 times, and more preferably from about 10 times to about 2,000 times, the oil phase volume.

The above-described emulsifier and osmotic pressure regulator that may be added to the external water phase, and the subsequent procedures are the same as those described in paragraph (I) above.

Production methods for sustained-release compositions of the present invention, which contain a biologically active substance, a hydroxynaphthoic acid or salt thereof,

and a biodegradable polymer, e.g., microspheres, are exemplified below.

(I) Aqueous drying method

(i) O/W method

A biologically active substance or salt thereof is added to a solution of a hydroxynaphthoic acid and a biodegradable polymer in an organic solvent to a weight ratio falling within the above-described content range for biologically active substances, to yield an organic solvent solution of the biologically active substance, hydroxynaphthoic acid and biodegradable polymer.

Said organic solvent is exemplified by halogenated hydrocarbons (e.g., dichloromethane, chloroform, dichloroethane, trichloroethane, carbon tetrachloride), ethers (e.g., ethyl ether, isopropyl ether), fatty acid esters (e.g., ethyl acetate, butyl acetate), aromatic hydrocarbons (e.g., benzene, toluene, xylene), alcohols (e.g., ethanol, methanol), and acetonitrile. These solvents may be used in mixtures at appropriate ratios. Of these solvents, mixtures of halogenated hydrocarbons and alcohols are preferred, with greater preference given to mixtures of dichloromethane and ethanol.

The biodegradable polymer concentration in the organic solvent solution varies depending on the molecular weight of biodegradable polymer and the kind of organic solvent. For example, when the organic solvent used is dichloromethane, the biodegradable polymer concentration is normally chosen over the range from about 0.5 to about 70% by weight, preferably from about 1 to about 60% by weight, and more preferably from about 2 to about 50% by weight.

When the organic solvent used is a mixture of dichloromethane and ethanol, the ratio of their concentrations is normally chosen over the range from about 0.01 to about 50% (v/v), preferably from about 0.05 to about 40% (v/v), and more preferably from about 0.1 to about 30% (v/v).

The thus-obtained organic solvent solution containing

a biologically active substance or salt thereof, a hydroxynaphthoic acid or salt thereof, and a biodegradable polymer, is then added to a water phase to form an O (oil phase)/W (water phase) emulsion, after which the solvent is evaporated from the oil phase to yield microspheres. For this operation, the water phase volume is normally chosen over the range from about 1 time to about 10,000 times, preferably from about 5 times to about 50,000 times, and more preferably from about 10 times to about 2,000 times, the oil phase volume.

An emulsifier may be added to the above-described external water phase. Said emulsifier may be any one, as long as it is capable of forming a stable O/W emulsion. Such emulsifiers include, for example, anionic surfactants (e.g., sodium oleate, sodium stearate, sodium lauryl sulfate), nonionic surfactants [e.g., polyoxyethylene sorbitan fatty acid esters (Tween 80, Tween 60, Atlas Powder Company), polyoxyethylene castor oil derivatives (e.g., HCO-60, HCO-50, Nikko Chemicals)], polyvinylpyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin and hyaluronic acid. These emulsifiers may be used singly or in combination. Regarding the concentration, it is preferable that they be used over the range from about 0.01% to 10% by weight, preferably from about 0.05% to about 5% by weight.

An osmotic pressure regulator may be added to the above-described external water phase. Said osmotic pressure regulator may be any one, as long as it shows an osmotic pressure when prepared as an aqueous solution.

Said osmotic pressure regulator is exemplified by polyhydric alcohols, monohydric alcohols, monosaccharides, disaccharides, oligosaccharides, amino acids, and derivatives thereof.

Useful polyhydric alcohols include, for example, dihydric alcohols such as glycerol, pentahydric alcohols

such as arabitol, xylitol and adonitol, and hexahydric alcohols such as mannitol, sorbitol and dulcitol. Of these alcohols, hexavalent alcohols are preferred, with greater preference given to mannitol.

Useful monohydric alcohols include, for example, methanol, ethanol and isopropyl alcohol, with preference given to ethanol.

Useful monosaccharides include, for example, pentoses such as arabinose, xylose, ribose and 2-deoxyribose, and hexoses such as glucose, fructose, galactose, mannose, sorbose, rhamnose and fucose, with preference given to pentoses.

Useful oligosaccharides include, for example, trisaccharides such as maltotriose and raffinose, and tetrasaccharides such as stachyose, with preference given to trisaccharides.

Useful derivatives of monosaccharides, disaccharides and oligosaccharides include, for example, glucosamine, galactosamine, glucuronic acid and galacturonic acid.

Useful amino acids include, for example, glycine, leucine and arginine, with preference given to L-arginine.

These osmotic pressure regulators may be used singly, or in combination.

These osmotic pressure regulators are normally used at such concentrations that the external water phase osmotic pressure is about 1/50 to about 5 times, preferably about 1/25 to about 3 times, the physiological saline osmotic pressure.

Organic solvent removal can be achieved by commonly known methods or methods based thereon. Such methods include, for example, the method in which the organic solvent is evaporated under normal or gradually reduced pressure during stirring using a propeller stirrer, magnetic stirrer or the like, and the method in which the organic solvent is evaporated while the degree of vacuum is

adjusted using a rotary evaporator or the like.

The thus-obtained microspheres are centrifuged or filtered to separate them, after which they are washed with distilled water several times to remove the free biologically active substance, hydroxynaphthoic acid, drug support, emulsifier etc. adhering to the microsphere surface, then again dispersed in distilled water etc. and freeze-dried.

To prevent mutual aggregation of particles during the production process, an anticoagulant may be added. Said anticoagulant is exemplified by water-soluble polysaccharides such as mannitol, lactose, glucose and starches (e.g., corn starch), amino acids such as glycine, and proteins such as fibrin and collagen. Of these substances, mannitol is preferred.

Where necessary, freeze-drying may be followed by heating under reduced pressure without causing mutual adhesion of microspheres, to remove the water and organic solvent from the microspheres. It is preferable that the microspheres be heated at a temperature slightly higher than the intermediate glass transition point of the biodegradable polymer, as determined using a differential scanning calorimeter when the temperature is increased at a rate of 10 to 20°C per minute. More preferably, the microspheres are heated within the temperature range from the intermediate glass transition point of the biodegradable polymer to a temperature higher by about 30°C than the glass transition temperature. When a lactic acid-glycolic acid polymer is used as the biodegradable polymer, in particular, it is preferable that the microspheres be heated within the temperature range from the intermediate glass transition point to a temperature higher by 10°C than the glass transition temperature, more preferably within the temperature range from the intermediate glass transition point to a temperature higher by 5°C than the glass transi-

tion temperature.

Although it varies depending on the amount of microspheres and other factors, heating time is normally about 12 hours to about 168 hours, preferably about 24 hours to about 120 hours, and more preferably about 48 hours to about 96 hours, after the microspheres reach a given temperature.

Any heating method can be used, as long as microsphere aggregates are uniformly heated.

Useful thermal drying methods include, for example, the method in which thermal drying is conducted in a constant-temperature chamber, fluidized bed chamber, mobile chamber or kiln, and the method using microwaves for thermal drying. Of these methods, the method in which thermal drying is conducted in a constant-temperature chamber is preferred.

(ii) W/O/W method (1)

A biologically active substance or salt thereof is added to a solution of a biodegradable polymer in an organic solvent to a weight ratio falling within the above-described content range for biologically active substances, to yield an organic solvent solution of the biologically active substance and biodegradable polymer.

Said organic solvent is exemplified by halogenated hydrocarbons (e.g., dichloromethane, chloroform, dichloroethane, trichloroethane, carbon tetrachloride), ethers (e.g., ethyl ether, isopropyl ether), fatty acid esters (e.g., ethyl acetate, butyl acetate), aromatic hydrocarbons (e.g., benzene, toluene, xylene), alcohols (e.g., ethanol, methanol), and acetonitrile. These solvents may be used in mixtures at appropriate ratios. Of these solvents, halogenated hydrocarbons are preferred, with greater preference given to dichloromethane.

The biodegradable polymer concentration in the organic solvent solution varies depending on the molecular

weight of biodegradable polymer and the kind of organic solvent. For example, when the organic solvent used is dichloromethane, the biodegradable polymer concentration is normally chosen over the range from about 0.5 to about 70% by weight, preferably from about 1 to about 60% by weight, and more preferably from about 2 to about 50% by weight.

Next, to the organic solvent solution (oil phase) of the biologically active substance and biodegradable polymer, a solution of a hydroxynaphthoic acid or salt thereof [e.g., alkali metal salts (sodium salt, potassium salt etc.), alkaline earth metal salts (e.g., calcium salt, magnesium salt), or salts with transition metals (e.g., zinc, iron, copper)] [this solvent exemplified by water, alcohols (e.g., methanol, ethanol), pyridine, dimethylacetamide etc.] is added. This mixture is emulsified by a known method such as homogenization or sonication to form a W/O emulsion.

The thus-obtained W/O emulsion containing a biologically active substance, hydroxynaphthoic acid, and a biodegradable polymer, is then added to a water phase to form a W (internal water phase)/O (oil phase)/W (external water phase) emulsion, after which the solvent is evaporated from the oil phase to yield microspheres. For this operation, the external water phase volume is normally chosen over the range from about 1 time to about 10,000 times, preferably from about 5 times to about 50,000 times, and more preferably from about 10 times to about 2,000 times, the oil phase volume.

The above-described emulsifier and osmotic pressure regulator that may be added to the external water phase, and the subsequent procedures are the same as those described in paragraph (I) (i) above.

(ii) W/O/W method (2)

A biologically active substance or salt thereof [e.g., alkali metal salts (sodium salt, potassium salt etc.), al-

kaline earth metal salts (e.g., calcium salt, magnesium salt), or salts with transition metals (e.g., zinc, iron, copper)] is added to a solution of a biodegradable polymer in an organic solvent to a weight ratio falling within the above-described content range for hydroxynaphthoic acids, to yield an organic solvent solution of the hydroxynaphthoic acid and biodegradable polymer.

Said organic solvent is exemplified by halogenated hydrocarbons (e.g., dichloromethane, chloroform, dichloroethane, trichloroethane, carbon tetrachloride), ethers (e.g., ethyl ether, isopropyl ether), fatty acid esters (e.g., ethyl acetate, butyl acetate), aromatic hydrocarbons (e.g., benzene, toluene, xylene), alcohols (e.g., ethanol, methanol), and acetonitrile. These solvents may be used in mixtures at appropriate ratios. Of these solvents, mixtures of halogenated hydrocarbons and alcohols are preferred, with greater preference given to mixtures of dichloromethane and ethanol.

The biodegradable polymer concentration in the organic solvent solution varies depending on the molecular weight of biodegradable polymer and the kind of organic solvent. For example, when the organic solvent used is dichloromethane, the biodegradable polymer concentration is normally chosen over the range from about 0.5 to about 70% by weight, preferably from about 1 to about 60% by weight, and more preferably from about 2 to about 50% by weight.

Next, to the organic solvent solution (oil phase) of the hydroxynaphthoic acid and biodegradable polymer, a solution of a biologically active substance or salt thereof [this solvent exemplified by water, alcohols (e.g., methanol, ethanol)] is added. This mixture is emulsified by a known method such as homogenization or sonication to form a W/O emulsion.

The thus-obtained W/O emulsion containing a biologically active substance, hydroxynaphthoic acid, and a biodegradable polymer, is then added to a water phase to form

a W (internal water phase)/O (oil phase)/W (external water phase) emulsion, after which the solvent is evaporated from the oil phase to yield microspheres. For this operation, the external water phase volume is normally chosen over the range from about 1 time to about 10,000 times, preferably from about 5 times to about 50,000 times, and more preferably from about 10 times to about 2,000 times, the oil phase volume.

The above-described emulsifier and osmotic pressure regulator that may be added to the external water phase, and the subsequent procedures are the same as those described in paragraph (I) (i) above.

(II) Phase separation method

For producing microspheres by this method, a coacervating agent is added little by little to the organic solvent solution described in aqueous drying method paragraph (I) above, which contains a composition consisting of a biologically active substance, hydroxynaphthoic acid and biodegradable polymer, during stirring, to precipitate and solidify the microspheres. Said coacervating agent is added in an amount by volume of about 0.01 to 1,000 times, preferably about 0.05 to 500 times, and more preferably about 0.1 to 200 times, the oil phase volume.

Said coacervating agent may be any one, as long as it is a polymer, mineral oil or vegetable oil compound that is miscible in the organic solvent, and that does not dissolve the salt complex of the biologically active substance with the hydroxynaphthoic acid and biocompatible polymer. Specifically, useful coacervating agents include, for example, silicon oil, sesame oil, soybean oil, corn oil, cotton seed oil, coconut oil, linseed oil, mineral oil, n-hexane and n-heptane. These may be used in combination.

The microspheres thus obtained are collected, after which they are repeatedly washed with heptane etc. to re-

move the coacervating agent etc. other than the composition of the biologically active substance, hydroxynaphthoic acid and biodegradable polymer, followed by drying under reduced pressure. Alternatively, the microspheres are washed in the same manner as in aqueous drying method paragraph (I) (i) above, then freeze-dried and thermally dried.

(III) Spray drying method

For producing microspheres by this method, the organic solvent solution described in aqueous drying method paragraph (I) above, which contains a composition consisting of a biologically active substance, hydroxynaphthoic acid and biodegradable polymer, is sprayed via a nozzle into the drying chamber of a spray drier to volatilize the organic solvent in the fine droplets in a very short time, to yield microspheres. Said nozzle is exemplified by the double-fluid nozzle, pressure nozzle and rotary disc nozzle. The microspheres may be then freeze-dried and thermally dried as necessary after being washed in the same manner as that described in aqueous drying method paragraph (I) above.

For a dosage form other than the above-described microspheres, the organic solvent solution described in aqueous drying method paragraph (I) above, which contains a composition consisting of a biologically active substance, hydroxynaphthoic acid and biodegradable polymer, may be dried by evaporating the organic solvent and water, while the degree of vacuum is adjusted using a rotary evaporator or the like, followed by milling with a jet mill or the like, to yield microparticles.

The milled microparticles may be then freeze-dried and thermally dried after being washed in the same manner as that described in aqueous drying method paragraph (I) for microsphere production.

The microspheres or microparticles thus obtained en-

able drug release corresponding to the rate of decomposition of the biodegradable polymer or lactic acid-glycolic acid polymer used.

The sustained-release composition of the present invention can be administered as such or in the form of various dosage forms prepared using it as a starting material, specifically as intramuscular, subcutaneous, visceral and other injectable preparations or implant preparations, nasal, rectal, uterine and other transdermal preparations, oral preparations [e.g., solid preparations such as capsules (e.g., hard capsules, soft capsules), granules and powders; liquids such as syrups, emulsions and suspensions] etc.

For example, the sustained-release composition of the present invention can be prepared as injectable preparations by suspending in water with a dispersing agent (e.g, surfactants such as Tween 80 and HCO-60, polysaccharides such as sodium hyaluronate, carboxymethyl cellulose and sodium alginate), a preservative (e.g., methyl paraben, propyl paraben), an isotonizing agent (e.g., sodium chloride, mannitol, sorbitol, glucose, proline) etc. to yield an aqueous suspension, or by dispersing in a vegetable oil such as sesame oil or corn oil to yield an oily suspension, whereby a practically useful sustained-release injectable preparation is obtained.

When the sustained-release composition of the present invention is used in the form of an injectable suspension, its particle diameter is chosen over a such range that the requirements concerning the degree of dispersion and needle passage are met. For example, the mean particle diameter normally ranges from about 0.1 to 300 nm, preferably from about 0.5 to 150 nm, and more preferably from about 1 to 100 nm.

The sustained-release composition of the present invention can be prepared as a sterile preparation by such

methods as the method in which the entire production process is aseptic, the method using gamma rays for sterilization, and the method in which a preservative is added, which methods are not to be construed as limitative.

Because of low toxicity, the sustained-release composition of the present invention can be used as a safe pharmaceutical etc. in mammals (e.g., humans, bovines, swines, dogs, cats, mice, rats, rabbits).

Although varying widely depending on kind, content and dosage form of the active ingredient biologically active substance, and duration of release of the biologically active substance, target disease, subject animal species and other factors, the dose of the sustained-release composition may be set at any level, as long as the biologically active substance is effective. The dose of the active ingredient biologically active substance per administration can be preferably chosen as appropriate over the range from about 0.01 mg to 10 mg/kg body weight, more preferably from about 0.05 mg to 5 mg/kg body weight, per adult in the case of a 1-month release preparation.

The dose of the sustained-release composition per administration can be preferably chosen as appropriate over the range from about 0.05 mg to 50 mg/kg body weight, more preferably from about 0.1 mg to 30 mg/kg body weight per adult.

The frequency of administration can be chosen as appropriate, depending on kind, content and dosage form of the active ingredient biologically active substance, duration of release of the biologically active substance, target disease, subject animal species and other factors, e.g., once every several weeks, one every month or once every several months (e.g., 3 months, 4 months, 6 months).

[Examples]

The present invention is hereinafter described in

more detail by means of the following examples, which are not to be construed as limitative.

Example 1

3,429.6 mg of the acetate (produced by TAP) of N-(S)-tetrahydrofur-2-oyl-Gly-D2Nal-D4ClPhe-D3Pal-Ser-NMeTyr-DLys(Nic)-Leu-Lys(Nisp)-Pro-DAlaNH_2 (hereinafter referred to as peptide A) and 685.2 mg of 3-hydroxy-2-naphthoic acid were dissolved in 15 ml of ethanol. This solution was gradually distilled by means of a rotary evaporator to evaporate the organic solvent. This residue was again dissolved in 5.5 ml of dichloromethane and poured in 400 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry), previously adjusted to 18°C; the solution was stirred at 8,000 rpm, using a turbine type homomixer, to yield an O/W emulsion. This O/W emulsion was stirred at room temperature for 3 hours to volatilize the dichloromethane and solidify the oil phase, followed by microsphere collection at 2,000 rpm using a centrifuge (05PR-22, Hitachi, Ltd.). The microspheres were again dispersed in distilled water, after which centrifugation was conducted, and the free drug etc. washed down. The microspheres collected were again dispersed in a small amount of distilled water, then freeze-dried, to yield a powder. The recovery rate was 65%, and the peptide A content and 3-hydroxy-2-naphthoic acid/peptide A molar ratio in the microspheres were 75.4% and 1.94, respectively.

Example 2

1,785.1 mg of the acetate of peptide A and 1,370.4 mg of 3-hydroxy-2-naphthoic acid were dissolved in 15 ml of ethanol. This solution was gradually distilled by means of a rotary evaporator to evaporate the organic solvent. This residue was again dissolved in 10 ml of dichloromethane and poured in 1,000 ml of a 0.1% (w/w) aqueous solution of

polyvinyl alcohol, previously adjusted to 18°C; the same procedures as those in Example 1 were followed to yield microspheres. The recovery rate was 58%, the peptide A content and 3-hydroxy-2-naphthoic acid/peptide A molar ratio in the microspheres were 54.3% and 6.15, respectively.

Example 3

1,800 mg of the acetate of peptide A, 173 mg of 3-hydroxy-2-naphthoic acid, and 2 g of a lactic acid-glycolic acid copolymer (lactic acid/glycolic acid = 50/50 (mol%), weight-average molecular weight 10,100, number-average molecular weight 5,670, number-average molecular weight 3,720, as determined by terminal group quantitation, produced by Wako Pure Chemical Industries) were dissolved in a mixture of 6 ml of dichloromethane and 0.2 ml of ethanol. This solution was poured into 900 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol containing 5% mannitol, previously adjusted to 18°C, and stirred at 7,000 rpm using a turbine type homomixer to yield an O/W emulsion. This O/W emulsion was stirred at room temperature for 3 hours to volatilize the dichloromethane and ethanol and solidify the oil phase, followed by microsphere collection at 2,000 rpm using a centrifuge. The microspheres were again dispersed in distilled water, after which centrifugation was conducted, and the free drug etc. washed down. The microspheres collected were again dispersed in 250 mg of mannitol and a small amount of distilled water, then freeze-dried, to yield a powder. The recovery rate was 76%, the rate of peptide A inclusion in the microspheres was 84.6%, and the peptide A content and 3-hydroxy-2-naphthoic acid/peptide A molar ratio in the microspheres were 34.7% and 1.19, respectively.

Example 4

1,900 mg of the acetate of peptide A, 182 mg of 3-hydroxy-2-naphthoic acid, and 1.9 g of a lactic acid-

glycolic acid copolymer (same as in Example 3) were dissolved in a mixture of 6 ml of dichloromethane and 0.2 ml of ethanol. This solution was poured in 900 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol containing 5% mannitol and 0.05% L-arginine, previously adjusted to 18°C; the same procedures as those in Example 3 were followed to yield microspheres. The recovery rate was 85%, the rate of peptide A inclusion in the microspheres was 88.9%, and the peptide A content and 3-hydroxy-2-naphthoic acid/peptide A molar ratio in the microspheres were 38.6% and 0.83, respectively.

Example 5

Microspheres were obtained in the same manner as in Example 4, except that the lactic acid-glycolic acid copolymer used in Example 4 was replaced with a lactic acid-glycolic acid copolymer having a lactic acid/glycolic acid content ratio of 75/25 (mol%), a weight-average molecular weight of 10,700, a number-average molecular weight of 6,100, and a number-average molecular weight of 3,770, as determined by terminal group quantitation, and that the amount of dichloromethane was changed to 6.5 ml. The recovery rate was 87%, the rate of peptide A inclusion in the microspheres was 88.3%, and the peptide A content and 3-hydroxy-2-naphthoic acid/peptide A molar ratio in the microspheres were 38.3% and 0.92, respectively.

Example 6

To a solution of 1,800 mg of the acetate of peptide A and 1.8 g of a lactic acid-glycolic acid copolymer (lactic acid/glycolic acid = 50/50 (mol%), weight-average molecular weight 12,700, number-average molecular weight 7,090, number-average molecular weight 4,780, as determined by terminal group quantitation, produced by Wako Pure Chemical Industries) in 7.2 ml of dichloromethane. To this solution, a solution of 196 mg of 3-hydroxy-2-naphthoic acid

sodium salt in 2.3 ml of water was added, followed by emulsification using a homogenizer, to yield a W/P emulsion. This emulsion was poured into 800 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol containing 5% mannitol, previously adjusted to 18°C, and stirred at 7,000 rpm using a turbine type homomixer to yield a W/O/W emulsion. The same procedures as those in Example 3 were followed to yield microspheres. The recovery rate was 79%, the rate of peptide A inclusion in the microspheres was 81.2%, and the peptide A content and 3-hydroxy-2-naphthoic acid/peptide A molar ratio in the microspheres were 32.8% and 0.91, respectively.

Example 7

A solution of 1,000 mg of the acetate (produced by TAP) of 5-oxo-Pro-His-Trp-Ser-Tyr-Dleu-Leu-Arg-Pro-NH-C_2H_5 (hereinafter referred to as peptide B) in 1 ml of distilled water was mixed with a solution of 150 mg of 3-hydroxy-2-naphthoic acid and 3.85 g of a lactic acid polymer (weight-average molecular weight 33,650, number-average molecular weight 20,120, number-average molecular weight 7,790, as determined by terminal group quantitation, produced by Wako Pure Chemical Industries) in a mixture of 5.5 ml of dichloromethane and 0.35 ml of ethanol. This mixture was emulsified, using a homogenizer, to yield a W/O emulsion. This W/O emulsion was then poured into 800 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol, previously adjusted to 18°C, and stirred at 7,000 rpm using a turbine type homomixer to yield a W/O/W emulsion. This W/O/W emulsion was stirred at room temperature for 3 hours to volatilize the dichloromethane and ethanol and solidify the oil phase, followed by microsphere collection at 2,000 rpm using a centrifuge. The microspheres were again dispersed in distilled water, after which centrifugation was conducted, and the free drug etc. washed down. The microspheres collected were again dispersed in a small amount

of distilled water, then freeze-dried, to yield a powder. The recovery rate was 49%, the rate of peptide B inclusion in the microspheres was 106.5%, and the peptide B content and 3-hydroxy-2-naphthoic acid/peptide B molar ratio in the microspheres were 21.3% and 0.93, respectively.

Experimental Example 1

About 40 mg of the microspheres obtained in each of Examples 1 and 2, or about 60 mg of the microspheres obtained in each of Examples 3 through 5, were dispersed in 0.5 ml of a dispersant (distilled water with 0.25 mg of carboxymethyl cellulose, 0.5 mg of Polysorbate 80, and 25 mg of mannitol, all dissolved therein), and subcutaneously administered to the backs of male SD rats at 8 to 10 weeks of age, using a 22G injection needle. After administration, each rat was killed, and the microspheres remaining at the administration site were taken and assayed for peptide A content. The results are shown in Table 1.

Table 1

	1 Day	1 Week	2 Weeks	3 Weeks	4 Weeks
Example 1	73%	30%	11%	6%	6%
Example 2	85%	37%	9%	1%	
Example 3	70%	31%	14%	9%	5%
Example 4	77%	29%	11%	10%	6%
Example 5	81%	44%	25%	17%	13%

Reference Example 1

A solution of 1,000 mg of the acetate of peptide B in 1 ml of distilled water was mixed with a solution of 4 g of a lactic acid polymer (weight-average molecular weight 33,650, number-average molecular weight 20,120, number-average molecular weight 7,790, as determined by terminal group quantitation, produced by Wako Pure Chemical Industries) in a mixture of 5 ml of dichloromethane. This mixture was emulsified, using a homogenizer, to yield a W/O

emulsion. This W/O emulsion was then poured into 800 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol, previously adjusted to 18°C, and stirred at 7,000 rpm using a turbine type homomixer to yield a W/O/W emulsion. This W/O/W emulsion was stirred at room temperature for 3 hours to volatilize the dichloromethane and ethanol and solidify the oil phase, followed by microsphere collection at 2,000 rpm using a centrifuge. The microspheres were again dispersed in distilled water, after which centrifugation was conducted, and the free drug etc. washed down. The microspheres collected were again dispersed in a small amount of distilled water, then freeze-dried, to yield a powder. The recovery rate was 49%, the rate of peptide B inclusion in the microspheres was 57.1%, and the peptide B content in the microspheres was 11.4%.

The experimental results of Examples 1 and 2 demonstrate that the rate of peptide A release from the microspheres consisting of two components, i.e., peptide A and 3-hydroxy-2-naphthoic acid, varied depending on their ratio; peptide A was more rapidly released as the 3-hydroxy-2-naphthoic acid content increased. Also, the experimental results in Examples 3, 4 and 5 demonstrate that the microspheres consisting of three components, i.e., the above two components and a lactic acid-glycolic acid copolymer, showed a peptide A release profile different from that from the microspheres consisting of the two. It was also shown that the release behavior of microspheres can be controlled by combining different lactic acid-glycolic acid copolymer compositions and weight-average molecular weights. The results in Example 7 and Reference Example 1 demonstrate that 3-hydroxy-2-naphthoic acid increases the peptide B content in microspheres.

[Effect of the invention]

The sustained-release composition of the present in-

vention contains a biologically active substance at high contents, and is capable of controlling the rate of its release.

Abstract of the Disclosure

[Summary]

[Object]

To provide a novel composition that contains a biologically active substance at high contents, and that is capable of controlling the rate of its release.

[Means of solving the problem]

A sustained-release composition containing a hydroxynaphthoic acid salt of a biologically active substance and a biodegradable polymer, a method of its production, and a pharmaceutical containing said sustained-release composition.

[Drawings selected]

None